Functional analysis of AVR3a of *Phytophthora infestans*, a member of the RXLR family of cytoplasmic effectors

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Abstract

Phytophthora infestans secretes two classes of effectors that are delivered to different cellular compartments of the host where they interact with plant targets to reprogram plant defenses. One class is secreted into the apoplast (apoplastic effectors), whereas the other is translocated inside the plant cell (cytoplasmic effectors). A combination of data mining and functional genomics facilitated the identification of AVR3a, a cytoplasmic effector of *P. infestans*. The AVR3a family is represented by at least two polymorphic secreted proteins that differ in two amino acids in the mature region. *P. infestans* isolates that are avirulent on *R3a* potato carry *Avr3a_K*^{80}*I*^{103}, whereas virulent isolates carry only *avr3a_E*^{80}*M*^{103}. Intracellular co-expression of *Avr3a_K*^{80}*I*^{103} and *R3a* by agroinfiltration in *Nicotiana benthamiana* resulted in the hypersensitive response (HR) suggesting that the two proteins interact in the plant cytoplasm. Indeed, in addition to a signal peptide, AVR3a contains the RXLR motif that is thought to promote the translocation of *Phytophthora* effectors inside host cells. We took advantage of the *N. benthamiana* assay to characterize the AVR3a effector and gain insight into the mechanisms that modulate host responses. Virus induced gene silencing showed that *R3a*-mediated HR requires the plant signaling components SGT1 and HSP90. We also showed that AVR3a suppresses the HR induced by *P. infestans* INF1 elicitin. Finally, deletion analyses revealed the functional domain of AVR3a. Altogether, this data shows that AVR3a exhibits both avirulence and cell death suppression functions.

Introduction

- ✓ *P. infestans* causes late blight on potato and tomato resulting in economic losses worldwide.
- ✓ We hypothesize that *P. infestans* delivers effectors to different cellular compartments of the host where they interact with plant targets to reprogram host defense and promote susceptibility.
- ✓ Armstrong *et. al.*, recently identified AVR3a, a cytoplasmic effector of *P. infestans* that contains an RXLR motif that is thought to be required for intracellular localization (Figure 1 and 2).
- ✓ The *Avr3* gene family is represented by at least two polymorphic members, *avr3a_S*^{19}*E*^{80}*M*^{103} and *Avr3a_C*^{19}*K*^{80}*I*^{103} that confer a virulent and avirulent phenotype on *R3a* in *Solanum demissum,* respectively.
- ✓ Co-expression of the recently cloned *R3a* gene with *Avr3a_K*^{80}*I*^{103} by agroinfiltration results in the HR in *N. benthamiana*.
- ✓ We aim to identify and characterize the AVR3a effector using expression assays in *N. benthamiana* to obtain a better understanding of the molecular mechanisms that reprogram host defense responses.

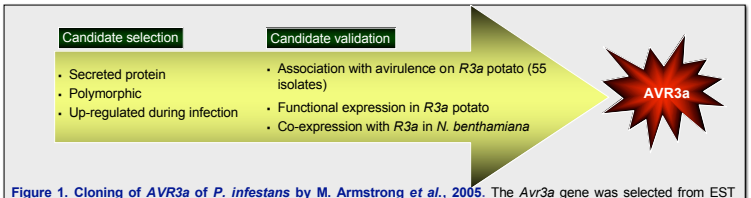

Figure 1. Cloning of *AVR3a* of *P. infestans* by M. Armstrong *et al.*, 2005. The *Avr3a* gene was selected from EST databases based on features shared by many avirulence genes, such as secretion, polymorphisms and up-regulation during infection. The avirulence function of AVR3a was identified using an association genetics approach (Bos *et al.*, 2003). Functional assays based on transient expression of *Avr3a* in potato and *N. benthamiana* confirmed its interaction with the resistance gene *R3a* from *Solanum demissum*.

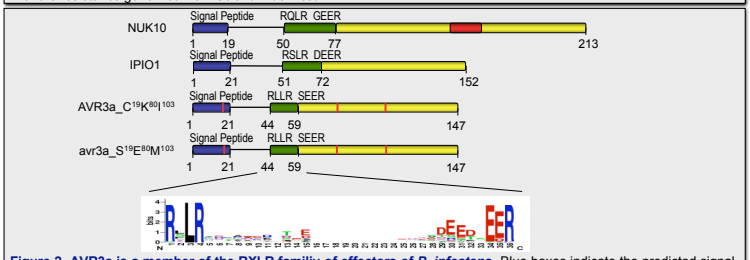

Figure 2. AVR3a is a member of the RXLR familiy of effectors of *P. infestans*. Blue boxes indicate the predicted signal peptide, green boxes the predicted RXLR-motif, yellow boxes the effector domain and the red box indicate a nuclear localization signal. The logo indicates the conserved RXLR motif and was obtained from a sequence alignment of 50 (candidate) effectors of *P. infestans*.

Results

❖ AVR3a is recognized by R3a upon co-expression in *N. benthamiana*

✓ Co-expression of *Avr3a_KI* and *R3a* using agroinfiltration resulted in HR, and thereby confirmed the interacting gene-pair (Figure 3).
✓ Co-expression of *avr3a_EM* and *R3a* resulted in weak recognition responses. Cell-death-associated autofluorescence was visible by UV fluorescent light microscopy (data not shown).

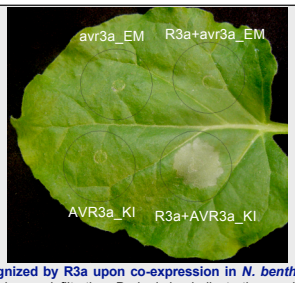

Figure 3. AVR3a is recognized by R3a upon co-expression in *N. benthamiana*. Co-expression of *R3a* and *Avr3a* was performed by agroinfiltration. Dark circles indicate the agroinfiltration sites. Infiltration of an *Agrobacterium* strain expressing *R3a* together with a strain expressing *Avr3a_KI* resulted in HR around 3-4 dpi. Infiltration of *Agrobacterium* strains expressing the *R3a* + *avr3a_EM* combination or infiltration of strains expressing *Avr3a_KI* or *avr3a_EM* alone did not result in HR.

❖ AVR3a-R3a mediated HR requires the plant signaling components SGT1 and HSP90

✓ *N. benthamiana* plants were silenced for a set of genes using virus induced gene silencing (VIGS). We performed agroinfiltration on these silenced plants with *Agrobacterium* strains expressing the *R3a* + *Avr3a_KI* combination.
✓ Silencing of SGT1 and HSP90 resulted in the loss of AVR3a-R3a mediated HR (Figure 4).

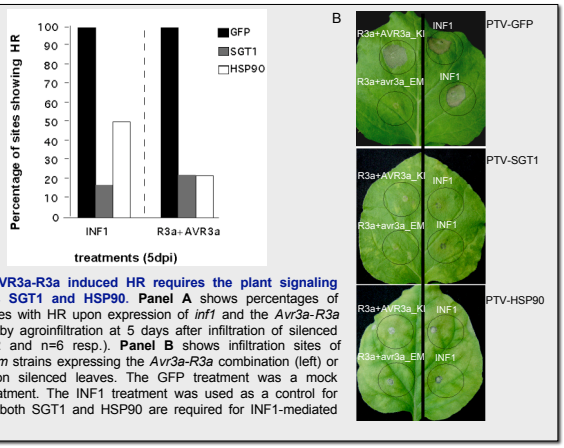

Figure 4. AVR3a-R3a induced HR requires the plant signaling components SGT1 and HSP90. Panel A shows percentages of infiltration sites with HR upon expression of *inf1* and the *Avr3a-R3a* combination by agroinfiltration at 5 days after infiltration of silenced plants (n=12 and n=6 resp.). **Panel B** shows infiltration sites of *Agrobacterium* strains expressing the *Avr3a-R3a* combination (left) or *inf1* (right) on silenced leaves. The GFP treatment was a mock silencing treatment. The INF1 treatment was used as a control for silencing as both SGT1 and HSP90 are required for INF1-mediated HR.

❖ AVR3a_KI suppresses the HR induced by *P. infestans* INF1 elicitin

✓Co-expression of the elicitin *inf*1 together with *Avr3a_KI* suppressed the INF1-mediated HR (Figure 5).

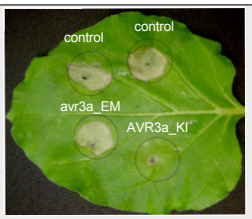

Figure 5. AVR3a_KI suppresses the HR induced by INF1 elicitin. Sites were agroinfiltrated with *Agrobacterium* strains expressing *Avr3a*, *avr3a* or *Δgfp* (control) and challenged 1 dpi with strains expressing the elicitin *inf1*.

❖ The AVR3a 75 amino acid C-terminal domain is sufficient for avirulence and suppressor functions

✓ Deletion analysis revealed the AVR3a domain sufficient for AVR3a effector functions (Figure 6).

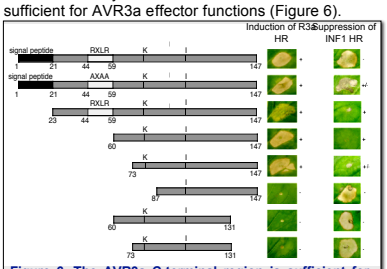

Figure 6. The AVR3a C-terminal region is sufficient for AVR3a effector functions. *Avr3a* (deletion) mutants were assayed by co-expression with *R3a* or *inf1* in *N. benthamiana* using agroinfiltration. Symptoms of HR were observed 2-5 days after infiltration.

Future work

- ✓ We will focus on the characterization of the virulence function of AVR3a.
  - –What are the interacting plant targets and how are plant responses manipulated ?
- ✓ We will characterize the structural requirements for AVR3a effector functions.
  - –AVR3a random mutagenesis (figure 7).

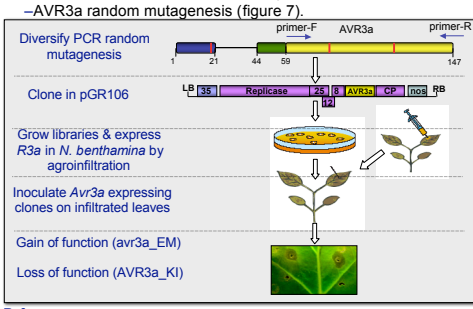

Figure 7. Random mutagenesis approach to identify key amino acids for AVR3a effector functions. The *Avr3a* gene is amplified using the Diversify PCR random mutagenesis kit from BD Sciences. Then PCR products are cloned in the pGR106 expression vector. Colonies are picked and grown for 2 days on LB agar plates. Leaf panels of *N. benthamiana* are infiltrated with *Agrobacterium* expressing *R3a*. One day after infiltrations leaf panels are toothpick inoculated with the mutant clones and symptoms are observed 10-15 dpi. Clones of interest are sequenced to determine the key amino acids for avirulence function. Clones of interest will also be tested for INF1 suppressor function (not shown).

References

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